INSTITUTO POLITÉCNICO DE LEIRIA

ESCOLA SUPERIOR DE TURISMO E TECNOLOGIA DO MAR

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Doutor Jorge Dias

Professor Raúl Bernardino

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# Resumo

Hoje em dia a industria da aquacultura enfrenta novos desafios para manter a sustentabilidade econômica de uma forma responsável para o meio ambiente e consumidores. Um dos maiores desafios que apresenta hoje em dia, é o aumento do preço da farinha e óleo de peixe que são os constituintes maioritários da alimentação para os peixes de aquacultura. As PAP's e gorduras de animais terrestres parecem ser uma alternativa viável, sustentável e menos problemática do que as farinhas e óleos vegetais para substituir a farinha e óleo de peixe. Por esta razão neste trabalho nos procuramos substituir uma pequena parte da farinha e óleo de peixe por alguns produtos provenientes de animais terrestres.

Este estudo foi um teste de crescimento para a truta arco-íris (*Oncorhynchus mykiss*). Inicialmente foram formuladas 4 dietas (CTRL, PAP2, PAP3 and PAP4). Exceptuando o dieta controlo (CTRL) que foi uma dieta comercial, os outros alimentos foram desenhados para substituir a farinha de peixe e outras farinhas vegetais por PAP's (proteína animal processada) como hidrolisado de farinha de penas, farinha de sangue de suíno e gordura animal processadas como a gordura de aves.

O teste foi feito ao longo de 112 dias com *checkpoints* no dia (30, 60 e 112), onde os peixes eram pesados com excepção do dia 112 onde foram também amostrados para composição corporal e índice hepatossomático. O peso inicial médio dos peixes dos grupos foi 219,7g e o peso médio final foi 488,7g. Não existiram alterações comportamentais nem preferência por nenhuma ração testada e não foram verificadas diferenças estatisticamente significativas (P>0,05) no consumo de ração, crescimento e outros parâmetros de crescimento com a exceção da taxa de eficiência proteica. Foram também verificadas diferenças estatísticas significativas para alguns aspectos da composição corporal, ganhos e retenções. Os resultados demonstram que as substituições feitas não tiveram impacto no crescimentos da truta arco íris mas sim na composição corporal. Apesar disso os resultados não sugerem nenhum problema importante relacionado com o uso de PAP's na formulação de ração para peixes.

**Palavras-Chave:** *Oncorhynchus mykiss*; PAP; proteína animal processada; gordura animal processada.

# Abstract

Nowadays the aquaculture industry faces new challenges to maintain the economic sustainability in a responsible way in relation to the environment and to the consumer. One of the major problems faced is the increase on fish meal and oil price which are the major components in the aquaculture feed formulation. PAP's and terrestrial animal fat seems to be the more viable, sustainable and less problematic alternative than vegetable products to replace the fish meal and oil. For this reason on this work we search to replace a little amount of fish and oil meal for some products from terrestrial animals.

This study was a growth trial with rainbow trout (*Oncorhynchus mykiss*). Initially were formulated 4 diets (CTRL, PAP2, PAP3 and PAP4). With the exception of the control diet (CTRL) that was a commercial diet, the other feeds were designed to replace fish meal and other vegetable meals by PAP's (Processed animal proteins) and processed animal fats such as feather meal hydrolisate, porcine blood meal and poultry fat.

The trial extended for 112 days with *checkpoints* at day (30, 60 and 112) where the fishes were weighed and in the end, they were also sampled for body composition and hepatosomatic index. The groups fishes average initial weight was 219.7g and the average final body weight was 488.7g. There were no behavioral differences in the preference for any of the feeds tested and were no significant statistical differences ($P>0.05$) on feed intake, growth and other growth parameters excepting the protein efficiency ratio. The fish body composition, gain and retention values also showed significant statistical differences for some aspects. The results shown that the substitutions made did not had an impact on rainbow trout growth but the body composition was affected by the feed composition. Nevertheless the results did not suggest any important issue related to the use of the PAP's in the fish feed formulation.

**Keywords:** *Oncorhynchus mykiss*; PAP; rendered animal protein; rendered animal fat.

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# Abbreviations and Acronyms

| | |
|---|---|
| AOAC | Association of Official Analytical Chemists |
| BM | Blood Meal |
| BSE | Bovine spongiform encephalopathy |
| DM | Dry matter |
| EN | Energy |
| FBW | Final Body Weight |
| FCR | Feed Conversion Rate |
| FeM | Feather Meal |
| HI | Hepatosomatic index |
| IBW | Initial Boy Weight |
| MBM | Meat Bone Meal |
| PAP | Processed animal Protein or Proteína animal processada |
| PBM | Poultry by Meal |
| PER | Protein Efficiency ratio |
| PRO | Protein |
| SGR | Specific growth ration |
| TSE | Transmissible spongiform encephalopathy |
| VFI | Voluntary feed intake |
| WG | Weight gain |

# 1. Introduction

## 1.1. Aquaculture in world and Europe

Nowadays we see an increase in advertising campaigns teaching the masses to eat healthy products such as fish. The population sensibilization to these matters lead to an increased search for sea products due to the high content in omega-3 and other polyunsaturated fatty acids, important in protection and prevention of some human diseases (Tacon & Metian 2008). Although this fact seems an improvement to society, some logistic problems were created. The fishery cannot sustain the demand because the fish stock in ocean has greatly diminished throughout the years and aquaculture seems to be the most reasonable solution to this issue (Jew et al. 2009).

Aquaculture is one of the fastest growing food producing sectors in the world (Bureau 2006; FAO 2010). The United Nations Food and Agriculture Organization estimated that the total production of cultured finfish, shell-fish and aquatic plants at 51 million metric tons (112 billion euros valued at 60 billion euros in 2003 (Bureau 2006). China is the largest single producer with reported 32.7 million tons, or 62% of the global aquaculture production (Bureau 2006; FAO 2011). Asia (excluding China) has retained its progressively dominant position in world aquaculture production, of approximately 26.1%, while America (4.60%), Europe (4.50%) and Africa (1.80%) account for the remaining major globe aquaculture producing regions by quantity (FAO 2010). Today, one third of the fish consumed by humans is produced by aquaculture with tendency to grow (Nengas et al. 1999; Bureau 2006; FAO 2011).

As for the rest of the world, aquaculture is European's fastest growing primary industry (FAO 2010). In 2008, European fish and shell fish farmers produced nearly 2.5 million tons worth 9.4 billion euros, with an average value of 3.73€/kg (Fao, 2011). Norway has by far the greatest impact on European aquaculture production (33.2%), followed by UK (10.2%), France (8.7%), Italy (8.6%), Greece (5.8%) and Spain (5.5%) (FAO 2010). Since 1990, freshwater aquaculture production dropped, whereas marine aquaculture increased due to the population preferences. This fact makes Europe the world leader in the production of some high value species (FAO 2010).

There is more than 200 species produced in aquaculture, mainly consisting in low value species like carp, catfish and milkfish especially in Asia. In Europe the main focus of production consists in high value carnivorous species like sea bream and bass, salmon, trout and eel (Bureau 2006; Tacon & Metian 2008).

### 1.2. Trout Aquaculture (*Oncorhynchus mykiss*)

Rainbow trout belongs to the Salmonidae family and is a native species from North America, although since the year of 1874 it has been introduced to waters on all continents except Antarctica, for recreational angling and aquaculture (Hardy 2002; Boujard et al. 2002).

Rainbow trout is capable of occupying many different habitats, ranging from an anadromous life history [strain known as steelhead] (living in the ocean but spawning in gravel-bottomed, fast-flowing, well-oxygenated rivers and streams) to permanently inhabiting lakes (Fornshell 2002; Hardy 2002).

Figure 3.1 - Rainbow Trout *Oncorhynchus mykiss* (Walbaum, 1792).

It is an easy fish to spawn and in the right conditions can achieve fast growing rates (Hardy 2002). This species tolerates temperatures between 0-27 °C and is dependent on the well oxygenated waters. Females are able to produce up to 2000 eggs/kg of body weight and normally spawn once in the Spring (January-May)(Hardy 2002). Trout will not spawn naturally in culture systems; thus, juveniles must be obtained either by artificial spawning in a hatchery or by collecting eggs from wild stocks (Fornshell 2002; Hardy 2002).

In aquaculture trout is produced mostly in intensive monocultures and requires high-quality water to meet a secure production. Feeds consist in compact nutritious pellets for all life stages, and need to have 35-45 percent of protein level and 16-22 percent of fat to achieve an optimal growth. Rainbow trout feeds use fish meal, fish oil, and grains (Hardy 2002). High energy diets can lead to conversion ratios of 1:1 (Boujard et al. 2002; Hardy 2002) The average production cost is between 1.06€ and 1.77€/kg (Fornshell 2002). The rainbow trout production has grown exponentially since the 1950s with almost 900.000 tons in the year 2012, particularly in Europe and, recently in Chile (Fornshell 2002). This is primarily due to an increased inland production in countries such as France, Italy, Denmark, Germany and Spain that aims to supply the domestic markets, and also mariculture, that is conducted in cages in Norway and Chile for the export market. Chile is currently the largest producer (Fornshell 2002; Hardy 2002)

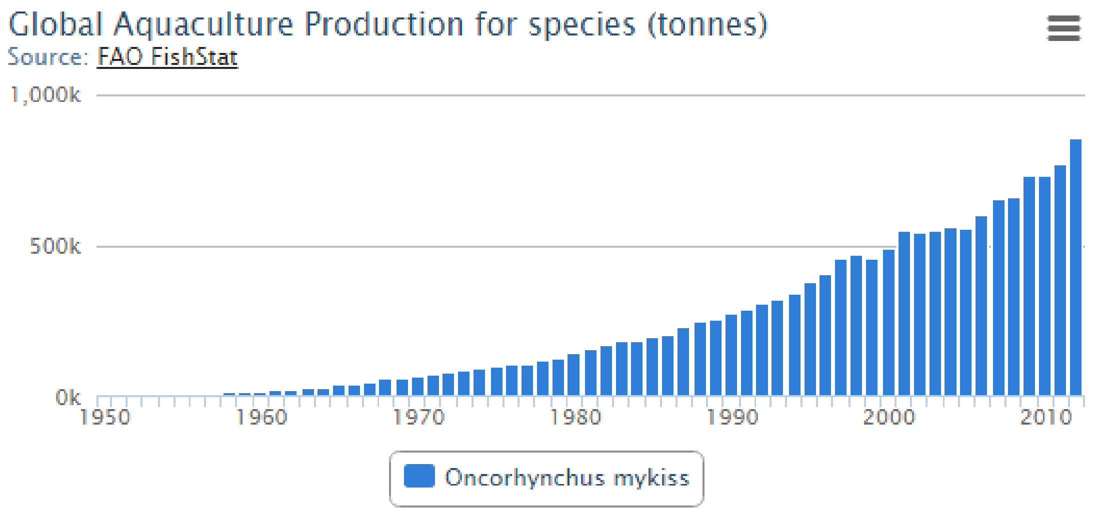



Figure 1.4 – Production in tons of *Oncorhynchus mykiss* until 2012.

The trout products for human consumption are presented as fresh, smoked, whole, filleted, canned, and frozen that are eaten steamed, fried, broiled, boiled, or micro-waved and baked. Trout processing wastes can be used for fish meal production or as fertilizer (Fornshell 2002). The fresh fish market is large because the flesh is soft and delicate, white to pink in color with a mild flavor. Food market fish size can be reached in 9 months but 'pan-sized' fish, generally 280-400 g, are harvested after 12-18 months. However, optimal harvest size varies globally: in the USA trouts are harvested at 450-600 g; in Europe at 1-2 kg; in Canada, Chile, Norway, Sweden and Finland at 3-5 kg (from marine cages)(Fornshell 2002). Preferences in meat color also vary globally with USA preferring white meat, but Europe and other parts of the world preferring pink meat generated from pigment supplements in aquafeed (Fornshell 2002; Hardy 2002).

### 1.3. Aquafeed necessity through the history

The main issue of the aquaculture industry is the aquafeed price representing about 45% of the variable costs of fish farms in Europe, mainly affected by is main components (fish meal, fish oil, krill, squid meal) and the manufacturing process used (extrusion, steam-pelleting) (Tomás et al. 2009; Bureau 2006). The typical cost estimated for aquafeed varies from 300€ to 1,500€ per metric ton dependent on species and life stages (Bureau 2006).

At this rate and with an increase in the world population, especially in sub developed countries, the aquaculture companies cannot keep expanding and producing, in a sustainable and profitable way, without improving some aspects of the industry, especially in aquafeed formulation (Nengas et al. 1999; Rana et al. 2009).

The fish meal and fish oil are highly digestible and provide all essential fatty acids (highly unsaturated omega-3 fatty acids), vitamins and amino acids important in the normal grow and quality of the fish. Because of this, since the beginning of aquaculture activity, up to the present date, the dependency for these components increased greatly (10% in 1988 to 46% in 2002) (Bureau et al. 2002; Tacon & Metian 2008) and the price had followed the trend due to natural sources over exploitation. This fact led to some ecological, ethical and economical concerns. To solve this problem, the industry explored other options with the aim of maintaining the profitability and sustainability (Bureau et al. 2002; Nengas et al. 1999; Tomás et al. 2009).

One of the proposed solutions was the full or partial substitution of fish meal and oil by vegetables protein and oils from soybean, rice, cereal and barley (Bureau 2006). In Europe, many studies were done in the most farmed species to improve the usage of these products and work around the problems imposed by them (Bureau 2006; Tacon & Metian 2008; Tomás et al. 2009). Most of the fish produced in Europe are carnivorous and the tolerance to vegetable products brings some digestion problems (Tomás et al. 2009). Vegetable products have strong anti-nutritional factors and yield low protein levels and lack on certain amino acids such methionine, lysine and tryptophan (Tomás et al. 2009). Despite all these facts, some studies successfully replaced great percentage of the fish meal/oil for vegetable meal/oil, but most of these studies lack the ability to avoid fish meal and oil inclusion, amino acid supplementation or expensive processes (Tacon & Metian 2008). The substitutions are highly dependent on the species and life stage and some treatments and inclusions had no economic viability (Martínez-Llorens et al. 2007; Tomás et al. 2009).

The search of vegetable products by other industries and human consumption led to an increase in the price of these products, mainly the soybean and cereal. This fact and the nutritional limitation in the vegetable products have imposed a new search for components to formulate aquafeeds to maintain the profitability (Tomás et al. 2009)

Rendered animal ingredients available in the market can be very useful for fish feed formulation and were proven to had a less quality and digestible problems than vegetable products (Tocher 2003; Emery et al. 2014). These ingredients were used in aquatic and terrestrial feeds for several decades (Bureau 2006). From the 1930s to the mid-1970s crude moist of proteins were included on feed for salmon and trout hatcheries in the USA and in Canada (Bureau 2006). These feeds were abandoned due to the lack of studies made, poor digestibility and quality problems (Tocher 2003; Bureau 2006). Another reason that led to the abandonment was the existence of some transmissible diseases like Bovine spongiform encephalopathy (BSE) and other Transmissible spongiform encephalopathies (TSEs). Luckily, with the pressure made by some industries and the decrease of these diseases prevalence , new norms were released by EU to liberate the conditional use of some animal proteins to be used in meals and feeds (Bureau et al. 2002; Tacon & Metian 2008; Hu et al. 2013). It is important to note that rendered animal ingredients derived by swine and poultry, and lipid ingredients such as tallow, have never been identified as being involved in BSE or TSE transmission (Bureau et al. 2002; Hu et al. 2013). To this day, many studies were made in this field and successfully improved the rendering and quality of these products, making possible their economic viability (Bureau 2006).

Despite all the scientific effort to improve the use of rendered terrestrial animal products in aquacultures feeds, it still necessary to research more and find better ways to use them.

There is wide range of rendered terrestrial animal products and therefore, many application possibilities in aquaculture (Bureau et al. 2002; Bureau 2006; Tacon & Metian 2008).

### 1.4. Processed animal protein (PAP's)

PAP (processed animal proteins), also known as rendered protein, are terrestrial animal by-products processed from recycled animals to be incorporated in terrestrial and aquatic feed (Bureau et al. 2002; Meeker 2009; Hu et al. 2013). There is a high variability of products such as poultry by-products meal, meat and bone meal, spray-dried blood meal and hydrolyzed feather meal (Hu et al. 2013). Some of these are considered to be potential alternative protein sources in aquatic animal feed due to their high protein content, interesting amino acid profiles, and lack of anti-nutritional factors (Meeker 2009; Hu et al. 2013).

The digestibility of these products is variable. One of the first studies with poultry by-product meals (PBM) showed a relatively low digestibility (Cho & Slinger 1979). More recent digestible trials, using the same methodology, revealed a digestibly around 90% (Bureau et al. 1999). This fact may prove the improvement made in this component processing to this day (Bureau 2006; Meeker 2009; Hu et al. 2013).

The general conclusion of some studies point 20 to 25 percent of PBM inclusion without affecting the growth and feed conversion in the salmonids (Bureau 2006; Meeker 2009). Nutrition value of PBM is very similar to fish meal especially for rainbow trout. Due to the similarity of fish meal and PBM nutritional value, it is estimated that is possible to replace all fish meal for PBM in rainbow trout without negatively impacting performance, but some studies suggests that is wiser to use lower levels of inclusion (30 percent) to provide a safer and excellent growth performance (Bureau et al. 1999).

The blood meal (BM) shown to had some digestible variation due to material processing. Blood proteins have some sensitivity to heat and the drying techniques may damage the protein. It is possible to achieve a high digestibility with spray-dried BM. Also the amino acids availability (e.g. lysine) may be variable with the processing treatments (Bureau 2006; El-Haroun & Bureau 2007; Meeker 2009). Nowadays spray and ring-dried BMs are widely used in salmonid feeds due to their very high digestibility and consistent quality (Bureau 2006). Normally inclusions between 8-20 percent of BM can lead to good performance in fish (EL-Haroun et al. 2009).

Feather meals (FeMs) like PBM had a relatively low digestibility when the first studies come out (58-62%) (Cho & Slinger 1979) but, in more recent studies made in the rainbow trout, showed that the digestibility coefficient is more satisfactory (77-86%). This fact was also verified in other species, such as rockfish (*Sebastes schlegeli*) (Lee 2002). FeM is quite commonly used in fish feeds at lower levels 5-10 percent. Moreover, some studies reveal that were possible incorporated 15 to 25 percent without a negative effect on growth efficiency (Bureau et al. 2000).

Meat and bone meal (MBM) is a component with variable protein digestibility and greatly depends on the provenience of the components (Bureau 2006; Meeker 2009; Hu et al. 2013). Some studies reported 83 to 89 percent of digestibility, on rainbow trout (Bureau et al. 1999), but other studies reported lower values (74 to 79 percent) for Silver perch (*Bidyanus bidyanus*) (Allan et al. 2000). It was observed that an inclusion of 24 percent of MBM was possible, with satisfying results, on rainbow trout (Bureau et al. 2000; EL-Haroun et al. 2009).

Combinations of high quality rendered animal proteins could replace most of the fish meal on rainbow trout diet with good performance (Bureau 2006; Hu et al. 2013). EL-Haroun et al (2009) made a study that formulates some diets using MBM ,PBM and FeM combinations constituting two-thirds of the digestible protein. PBM/FeM combination did not reveal statistically significant differences in the growth rate compared to fish fed on control diet, MB/ FeM and MBM/PBM diets had slightly lower growths. Despite this fact the results clearly indicate that most rendered animal protein ingridients have a high nutritive value and can be very valuable protein sources for fish feed formulation (EL-Haroun et al. 2009). Nontheless, feeds should be formulated on a digestible basis, and relatively conservative estimates of apparent digestibility or safety margins should be used. This is especially critical in the case of FeMs and MBMs (Bureau 2006; Meeker 2009; EL-Haroun et al. 2009; Hu et al. 2013).

## 1.5. Rendered animal fats

Rendered animal fats are a low cost sub product and can be used as alternative to fish oil. Like the PAP's is originated from recycled animals and slaughter by-products like trimmings, fat, bone, hide or even restaurant grease rendered in products as tallow, lard and yellow lard (Bureau et al. 2002; Tocher 2003; Emery et al. 2014). These widely available products are interesting alternatives to fish oil due to their high feed palatability and high nutrient digestibility, factors that vegetable oils cannot achieve (Bureau et al. 2002; Emery et al. 2014).

The fatty acid profile of rendered fats greatly differs from fish oil due to the ratio between saturated and unsaturated fatty acids and, even, the variability of the unsaturated fatty acids. These differences reflect changes in the fish body composition leading to higher levels of saturated fatty acids in the muscle (Bureau et al. 2002; Bureau 2006; Tocher 2003; Emery et al. 2014). These incorporations of fatty acids in the muscle are not strictly related to the amount of fatty acids in feeds and the response of the fish metabolism and incorporation may be variable (Tocher 2003; Emery et al. 2014). It is expected that the incorporation of small amount of rendered animal fats in the diet may have only marginal effect in flesh composition. In some cases, like in trout and coho salmon, the body lipid saturation only slightly increased, as the concentration of saturated fatty acid in the diet increase (Bureau et al. 2002; Emery et al. 2014).

The change prorogued by the rendered animal fats is a common concern among the fish producers, which fear a low quality product. Despite this, it is important to notice that the fatty acid composition in fish oils is highly variable and can also lead to a different lipid composition in produced fish (Bureau et al. 2002; Meeker 2009).

Another common concern is the taste of the fish. Some studies reveal a slight difference in the sensory attributes in fish, but the flavor, color and texture of fillets do not appear to be negatively affected by rendered animal fats incorporation in the diet (Bureau et al. 2002; Meeker 2009).

Studies report differences in the digestibility and nutritive value of lipid sources with different fatty acid profiles at different water temperatures, such as tallow and lard (Bureau et al. 2002; Bureau 2006; Meeker 2009). However, studies in rainbow trout do not reveal digestible differences when tallow was incorporated in combination with a certain amount of fish oil (Bureau et al. 2002; EL-Haroun et al. 2009).

Several studies have examined the use of poultry fat, tallow, and lard in the diet of various fish species and concluded that 30 to 40 percent of total lipids can be replaced without effects on growth performance. But It is also necessary to use animal fats with certain quality, containing all the essential fatty acids required by fish to allow proper digestibility and growth (Bureau et al. 2002; Bureau 2006).

## 1.6. Fish nutrition

### 1.6.1. Protein and amino acids

Carnivorous fish need high inclusion of proteins in their diets. This costly component plays an important role in amino acid contribution, important in growth processes and cell renewal in fish (Sanz et al. 2000). Generally, protein requirements for growing finfish, like trout, are typically between 35-55% of total dietary intake (Hardy 2002). The protein requirements are variable and dependent on the fish life stage, species, dietary protein quality, dietary amino acid composition and quantity of non-protein energy (Hardy 2002; Bureau 2006; Kaushik & Seiliez 2010). Protein in fish tissues is formed from all 20 major amino acids, but 10 of these amino acids cannot be synthesized and must be provided in the diet. The 10 essential amino acids are: arginine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, phenylalanine and valine (Hardy 2002; Webster & Lim 2002; Hu et al. 2013).

### 1.6.2. Lipids

Lipids play an important role in almost every physiological process, such as growth, health and reproduction (Bureau et al. 2002; Tocher 2003). The lipids are the provider of essential fatty acids (EFA) and energy to the fish. The energy given by the fatty acids contained in lipids are extremely important to the efficient protein utilization, absorption of fat-soluble vitamins and to regulate hormones (Bureau et al. 2002; Hardy 2002; Webster & Lim 2002). Most of the finfish cannot synthetize polyunsaturated fatty acids (PUFA) linoleic (18:2n-6) and linolenic acids (18:3n-3) (Arts et al. 2009; Bureau et al. 2002). For this reason they need to be provided in diet to achieve normal growth and development (Bureau et al. 2002; Lund 2007).

Fish PUFA requirements are linked to the ability of elongation and desaturation of fatty acids of 18-carbon. Therefore it is also necessary to provide highly unsaturated fatty acids (HUFA) such as eicosopentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), especially in marine fish feeds. Freshwater fishes have the ability to convert polyunsaturated fatty acids in HUFA so these normally are not included in their feeds (Arts et al. 2009).

Normally the requirement of dietary lipids level is correlated with the carcass of the fish (Tocher 2003). Nevertheless, high levels of lipids may be a problem in fish. Fish tend to store this lipids in the muscle and visceral cavity, leading to negative effects on the growth performance, product quality and shelf life (Hardy 2002; Webster & Lim 2002).

### 1.6.3. Carbohydrates

Fish do not have a specific dietary requirement for carbohydrates. Despite this fact, the presence of carbohydrates in feeds can result in less consumption of protein and lipid in the basal metabolic processes leading to a better growth efficiency (Trushenski et al. 2006) and also feed intake in some fishes like trout (Wilson 1994) and European seabass (Pérez et al. 1997).

Carbohydrates can be incorporated by cereal components on the herbivorous and omnivorous species with great tolerance values (40%) whereas in carnivorous diets is limited to 20% (Webster & Lim 2002). The inclusion value is also dependent on fish species, carbohydrate source, and the physical state of molecule and processing (Krogdahl et al. 2003).

Excessive amounts of carbohydrates in fish diets increase liver size and glycogen deposition in salmonids (Wilson 1994). Fish cannot digest fiber due to the lack of the enzyme cellulase and NSPases, thus only the presence of intestinal microbiota may partially digest it (Krogdahl et al. 2003). Excessive fiber in fish feeds has been shown to increase fecal output and reduce nutrient usage (Webster & Lim 2002).

Despite this, fibers are important binders and provide physical stability to the pallets, and generally the presence of 8% fiber in the diets can be tolerated (Webster & Lim 2002).

### 1.6.4. Vitamins

Vitamins are a group of organic compounds essential for growth, health and normal function in animals, that are required in small amounts and that have no structural functions or are used for energy purposes (Gatlin 2010; Webster & Lim 2002).

Fifteen vitamins have been shown to be essential in fish diets, eleven water-soluble (ascorbic acid, biotin, choline, folic acid, inositol, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine and vitamin B12) and 4 fat-soluble vitamins (vitamin A, D, E, and K). But the minimum requirements for all vitamins have been established for only a few species, such as channel catfish, common carp, seabream, trout and the Nile tilapia (Gatlin 2010; Webster & Lim 2002). Vitamin requirements are influenced by size, age, growth rate, nutrient interrelationships and environmental factors (Webster & Lim 2002; Estensoro et al. 2011). The deficiency of some vitamins can be related to reduced growth, lethargy, anemia, scoliosis, hemorrhages and mortality (Gatlin 2010). Vitamins are usually added to the feeds in the form of premixes at a rate of about 1-2% of total ingredients (Webster & Lim 2002).

### 1.6.5. Minerals

Fish need minerals for tissue formation, osmoregulation and other metabolic functions. Fish can absorb minerals not only from the diet but also from water (Gatlin 2010; Webster & Lim 2002).

Due to the high-content of minerals in salt water, marine fish normally do not suffer from mineral deficiencies. Dietary requirements have been established for macro minerals (calcium, phosphorous, magnesium, chloride, sodium, potassium and sulfur) and for micro minerals (cobalt, chromium, copper, iodine, iron, manganese, selenium and zinc) of a limited number of species (Gatlin 2010).

Dietary deficiencies of most minerals in fish include reduced growth rate, poor feed efficiency, bone demineralization, skeletal deformity, anorexia, erosion of fins, muscular dystrophy and thyroid hyperplasia (Gatlin 2010; Webster & Lim 2002). Furthermore, some micro minerals are potentially toxic when present in amounts higher than required. There is a major concern about the supplementation of some minerals, like phosphorous, that can lead to environmental eutrophication (Gatlin 2010). Like vitamins, minerals are added to the diets as mineral premix at a rate of about 1-2% of the total ingredients (Webster & Lim 2002).

## 1.7. Objective

This work emerges from the necessity to keep researching new solutions to aquafeeds and develop knowledge about terrestrial products used in fish aquafeeds. For this reason in this study we will assess the effect of various formulations with moderate levels of PAPs on the growth performance, feed utilization, body composition and environmental nutrient loads on rainbow trout.

# 2. Material and methods

## 2.1. Experimental diets

Four practical diets were formulated for rainbow trout (*Oncorhynchus mykiss*) containing 34-37% crude protein and 15-20% crude fat, with 19 kJ/g gross energy and covering the known nutritional requirements in vitamins, minerals and phosphorus for this species (Table2.1). The control diet (CTRL) was formulated without terrestrial meal and oil sources and include fish and vegetable meal and oil. Diets PAP2, PAP3 and PAP4 was formulated using terrestrial animal components such as FeM, Porcine BM and poultry fat in substitution of some of fish and vegetable meals and oil.

Main ingredients were grinded (below 250 µm) in a micropulverizer hammer mill (Hosokawa Alpine, model SH1, Germany). Powder ingredients were then mixed accordingly to the target formulation in a 90 L double-helix mixer to attain basal mixtures. No oils were incorporated at this stage. All diets were manufactured by extrusion (pellet size 4 mm) by means of a pilot-scale twin-screw extruder Clextral BC45 (Clextral, France) with a screw diameter of 55.5 mm and temperature ranging 105-110 ºC. Upon extrusion, all batches of extruded feeds were dried in a convection oven (OP 750-UF, LTE Scientifics, United Kingdom) for 2 h at 60 ºC. Following drying, pellets were allowed to cool at room temperature, and subsequently the oil was added under vacuum coating conditions in a vacuum mixer (PG-10VCLAB, Dinnisen, The Netherlands). Throughout the duration of the trial, experimental feeds were stored at room temperature. Samples of each diet were taken for proximate composition analysis.

## 2.2. Growth trial

All experiments were performed according to Federation of European Laboratory Animal Science Associations (FELASA) category C recommendations by trained scientists and following the European Directive 2010/63/EU of European Parliament and of the Council of European Union on the protection of animals used for scientific purposes.

**Table 2.1 -** Formulation and composition of the experimental diets.

| Ingredients (%) | CTRL | PAP2 | PAP3 | PAP4 |
|---|---|---|---|---|
| | % | % | % | % |
| Fishmeal 60 (Nacional) | 32.00 | 16.00 | 12.00 | 12.00 |
| Fish gelatin | 1.00 | 1.00 | 1.00 | 1.00 |
| Feathermeal hydrolisate | | 6.50 | 9.50 | 9.50 |
| Porcine blood meal | | 3.50 | 3.50 | 3.50 |
| Corn gluten | 7.00 | 4.00 | 6.40 | 3.80 |
| Soybean meal 48 | 18.00 | 15.00 | 18.00 | 15.00 |
| Rapeseed meal | 12.00 | 12.00 | 12.00 | 12.00 |
| Sunflower meal | 3.00 | | 3.00 | |
| Wheat meal | 13.10 | 16.30 | 10.50 | 15.60 |
| Wheat bran | | 5.00 | 5.00 | 5.00 |
| Fish oil - COPPENS | 3.60 | 4.04 | 2.70 | 4.16 |
| Soybean oil | 8.40 | 6.08 | 5.40 | 6.32 |
| Poultry fat | | 6.08 | 5.40 | 6.32 |
| Vit & Min[a] Premix PV01 | 1.00 | 1.00 | 1.00 | 1.00 |
| Binder (Kieselghur) | 0.30 | 0.30 | 0.30 | 0.30 |
| Antioxidant powder (Paramega) | 0.20 | 0.20 | 0.20 | 0.20 |
| Antioxidant liquid (Naturox) | 0.20 | 0.20 | 0.20 | 0.20 |
| Sodium propionate | 0.20 | 0.20 | 0.20 | 0.20 |
| Monocalcium phosphate | | 2.00 | 3.00 | 3.00 |
| L-Lysine | | 0.50 | 0.50 | 0.70 |
| DL-Methionine | | 0.10 | 0.20 | 0.20 |
| **Total** | **100.00** | **100.00** | **100.00** | **100.00** |

Premix, Portugal: Vitamins (mg or IU kg-1 diet): Vitamins (mg or IU kg-1 diet): Vitamin A (retinyl acetate), 20.000 UI; vitamin D3 (DL-cholecalciferol), 2000 UI; vitamin E (Lutavit E50), 100 mg; vitamin K3 (menadione sodium bisulfitete), 25 mg; vitamin B1(thiamine hydrochloride), 30 mg; vitamin B2 (riboflavin), 30 mg; calcium pantothenate, 100 mg; nicotinic acid, 200 mg; vitamin B6 (pyridoxine hydrochloride), 20 mg; vitamin B9 (folic acid), 15 mg; vitamin B12 (cyanocobalamin), 100 mg; vitamin H (biotin), 3000 mg; vitamin C (Lutavit C35), 1000 mg; inositol, 500 mg; colin chloride, 1000 mg; betaine (Betafin S1), 500 mg;

Minerals (mg or % kg-1 diet): Co (cobalt carbonate), 0.65 mg; Cu (cupric sulphate), 9 mg; Fe (iron sulphate), 6 mg; I (potassium iodide), 0.5 mg; Mn (manganese oxyde), 9.6 mg; Se (sodium selenite), 0.01 mg; Zn (zinc sulphate) 7.5 mg; Ca (calcium carbonate), 18.6%; KCl, 2.41%; NaCl, 4.0 %.

Rainbow trout (*Oncorhynchus mykiss*) was raised at the Experimental Research Station (Vila Real, Portugal) facilities of University of Trás-os-Montes e Alto Douro (UTAD). Eight homogeneous groups of 25 rainbow trout each with an average body weight of 219.7 ± 1.1 g, were randomly distributed in eight square fiberglass tanks (250 L capacity; water flow rate 120 L $h^{-1}$) in an open freshwater system (temperature: 11,5 ± 0.4 $C^o$ ; dissolved oxygen: 8.6 ± 0.6 mg $L^{-1}$; pH: 6.4 ± 0.2) with natural photoperiod. Ammonia and nitrite levels were monitored weekly using commercial kits and never exceeded 0.5 mg $L^{-1}$. Experimental diets were randomly assigned to the tanks establishing duplicate groups of fish per treatment that were hand-fed twice a day (10:00 am and 5:00 pm) until apparent satiation, during 112 days. Total feed consumption and mortality data were daily recorded for each tank. Every 4 weeks, fish were group weighed and biomass was used to calculate feed intake.

### 2.3. Sampling

At the beginning of the experiment fish were fasted for 24 h and group weighed. Six fish from the initial stock were sampled and stored for subsequent analysis of whole-body composition. To follow growth and feed utilization, the fish from each tank was bulk weighed every 30 days. Fish was deprived of feed for 12 hours before each weighting. At the end of the trial fish from each tank was individually weighted and measured for length. Six fish from each duplicate tank were sampled for whole-body composition analysis. All samples were freeze-dried until further analysis.

### 2.4. Analytical methods

All chemical analyses were performed according to AOAC procedures (AOAC, 1999) and samples were run in duplicates. Frozen whole body samples from each tank were pooled and minced without thawing, using a meat grinder before analysis. The experimental diets were finely milled and homogenized before analysis. Diets and carcass samples were analyzed for moisture content (105 °C for 24 h), ash content (550 °C for 6 h), crude protein (N×6.25, Leco N analyser, Model FP-528, Leco Corporation, St. Joseph, USA), crude lipid and gross energy determination (adiabatic bomb calorimeter, Werke C2000, IKA, Germany).

### 2.5. Calculations

Growth performance and feed utilization were studied in terms of final body weight (FBW, g), daily weight gain (DWG, %), specific growth rate (SGR, % body weight $day^{-1}$), feed intake (FI, % IBW $day^{-1}$), feed efficiency (FE), protein efficiency ratio (PER) and feed conversion ratio (FCR). In the used formulas, $W_0$ corresponds to the initial fish mean weights, $W_1$ to the final fish mean weights, and ln to the natural logarithm.

### 2.6. Statistical analysis

Data are presented as mean ± standard deviation and statistical analysis followed the methods outlined by Zar (1999). Data were tested for normality and homogeneity of variances using Kolmogorov-Smimov and Levene's test, respectively. Then, all data were subjected to one way analysis of variance (ANOVA) to test differences between dietary treatments. When these tests showed significance ($P < 0.05$), individual means were compared using Tukey's multiple comparison test. Significant differences were considered when $P \leq 0.05$. All statistical tests were performed using the Statgraphics Centurion XV statistical software (Statgraphics Inc., Virginia, USA).

# 3. Results

## 3.1. Diet analytics

**Table 3.1 –** Theoretical values of the feed formulation (protein, lipid, ash, phosphorous and energy).

| As fed basis | CTRL | PAP2 | PAP3 | PAP4 |
|---|---|---|---|---|
| Crude protein | 36.9 | 34.3 | 37.5 | 34.6 |
| Crude fat | 15.5 | 19.2 | 16.7 | 19.8 |
| Fiber | 3.1 | 3.0 | 3.6 | 3.0 |
| Starch | 10.0 | 13.6 | 10.6 | 13.2 |
| Gross Energy | 19.2 | 19.8 | 19.3 | 19.8 |
| Lys | 2.4 | 2.4 | 2.5 | 2.5 |
| Met + Cys | 1.4 | 1.3 | 1.5 | 1.4 |
| Total P | 1.6 | 1.5 | 1.6 | 1.6 |
| Ca | 2.8 | 2.0 | 2.0 | 1.9 |

The table also features the amino acid complementation and the values are represented in percentage.

Table 3.1 show slightly differences between feeds on protein, fat and energy values. It was implemented amino acid supplementation to obtain the protein profile needed for this specie.

**Table 3.2** – Real diet composition.

| | (%) | (% DM) | (% DM) | (% DM) | (% DM) | (kJ/g DM) |
|---|---|---|---|---|---|---|
| **Diets** | **DM** | **Protein** | **Lipid** | **Ash** | **Phosphorus** | **Energy** |
| **CTRL** | 89.2±0.0 | 40.6±0.0 | 17.1±0.0 | 15.2±0.2 | 1,8±0.0 | 24,1±0.1 |
| **PAP2** | 91.5±0.0 | 37.8±0.0 | 21.7±0.5 | 11.1±0.0 | 1,5±0.0 | 21,6±0.0 |
| **PAP3** | 91.5±0.0 | 41.2±0.3 | 18.8±0.1 | 10.8±0.0 | 1,7±0.0 | 21,5±0.1 |
| **PAP4** | 91.8±0.2 | 38.6±0.3 | 22.4±0.1 | 10.1±0.3 | 1,6±0.0 | 22,0±0.1 |

The table show the average value of (protein, lipid, ash, phosphorous and energy) after manufacturing. The values were obtained in relation to the percentage of dry matter (DM) and present standard deviation (n=2).

Table 3.2 show differences in protein, lipid, ash and energy between diets. These differences were also visible in the theoretical formulation (table 3.1). Some values slightly increase between the theoretical (table 3.1) and post processed feed (table 3.2).

The table indicates that the feeds were not isoproteic isolipidic and isoenergetic as were previewed.

## 3.2. Growth an feed efficiency

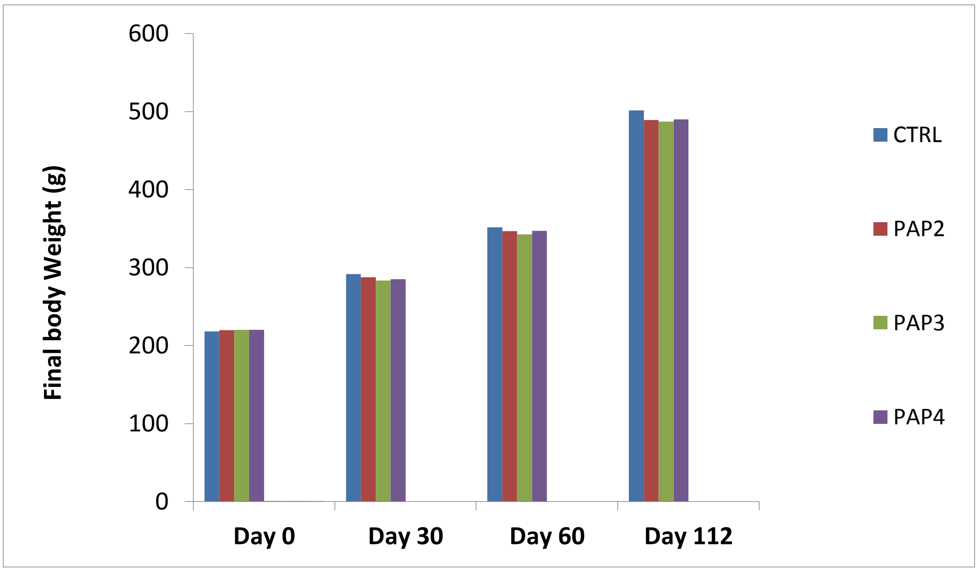
**Table 3.3 –** Growth parameters, feed efficiency, and hepatosomatic index after 112 days of trial.

| Diet | CTRL | PAP2 | PAP3 | PAP4 |
|---|---|---|---|---|
| **IBW** | 218.30 ± 0.42 | 220.00 ± 0,57 | 220.10 ± 1,27 | 220.30 ± 2,12 |
| **FBW** | 501.60 ± 4.53 | 489.20 ± 1.70 | 487.30 ± 5.52 | 490.00 ± 7.35 |
| **WG** | 1.16 ± 0.02 | 1.09 ± 0.01 | 1.08 ± 0.01 | 1.09 ± 0.05 |
| **SGR** | 0.74 ± 0.01 | 0.71 ± 0.01 | 0.71 ± 0.00 | 0.71 ± 0.02 |
| **FCR** | 1.05 ± 0.02 | 1.07 ± 0.01 | 1.09 ± 0.02 | 1.08 ± 0.04 |
| **VFI** | 0.73 ± 0.00 | 0.72 ± 0.00 | 0.73 ± 0.01 | 0.74 ± 0.01 |
| **PER** | 2.64 ± 0.04[ab] | 2.71 ± 0.02[a] | 2.44 ± 0.04[b] | 2.61 ± 0.11[ab] |
| **HI** | 0.92 ± 0.09 | 0.88 ± 0.08 | 0.87 ± 0.09 | 0.90 ± 0.11 |

The values represent the average and the standard deviation for each trial (n=2). Initial body weight (g) (IBM), Final body weight (g) (FBW), weight gain (%IBW/day) (WG), specific growth rate (%/day) (SGR), Feed conversion rate (FCR) (kg feed dry matter intake per kg live mass gain), Voluntary feed intake (VFI) (%/day), Protein efficiency ratio (PER) (gain in body mass per protein intake) and Hepatosomatic index (HI) (body mass per liver mass).

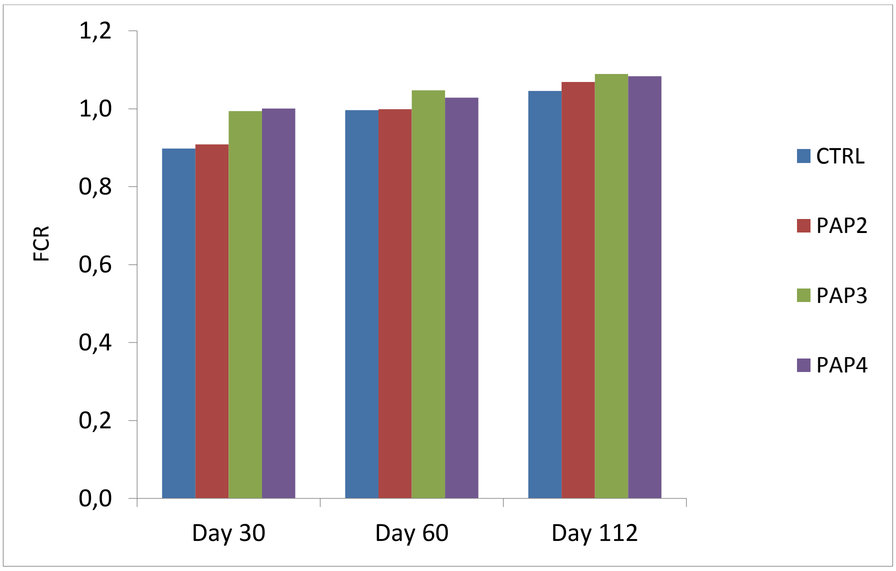
There was no mortality during the essay. The results of table 4 do not show significant statistical differences (P>0.05) between the trials as exception in protein efficiency rate parameter where CTRL and PAP3 showed statistical differences between the trials (table 3.3).

Graphic 3.1 reveal a uniform growth of the average weight during the trial checkpoints (day 0; 30; 60 and 112) and no significant statistical differences were found (P>0.05). The fish average initial body weight was 219.7g and the average final body weight was 488.7g. In general all the fishes on trial almost triplicate their weight after 112 days.




**Graphic 3.1 -** Average body weight evolution during the 112 days trial.

Graphic 3.2 represent the feed conversion rate evolution. The values do not show significant statistical differences (P>0.05) during the trail checkpoints (day 30; 60; 112). Day 30 had lower average values (0.95) and the day 112 had higher average values (1.07).




**Graphic 3.2 –** Feed conversion rate evolution during the 112 days trial

### 3.3. Fish condition

**Table 3.4 –** Fish corporal composition parameters in percentage.

| Corporal composition | CTRL | PAP2 | PAP3 | PAP4 |
|---|---|---|---|---|
| **DM %** | 99.04±0.42 | 98.54±0.65 | 98.08±0.44 | 98.00±0.54 |
| **ASH%** | 8.55±0.37[ab] | 8.62±0.55[ab] | 8.87±0.43[a] | 8.15±0.48[b] |
| **PRO%** | 61.35±0.88 | 59.88±1.87 | 62.96±1.49 | 58.29±2.41 |
| **FAT%** | 30.47±1.02[ab] | 31.16±1.56[ab] | 28.15±1.5[b] | 32.75±1.59[a] |
| **EN%** | 23.43±0.14 | 23.39±0.12 | 22.91±0.15 | 23.65±0.11 |

The table features each group average values for corporal composition parameters (dry matter (DM), ash (ASH), Protein (PRO) and lipids (FAT) and energy (EN)) after the 112 days trial with standard deviation (n=2) and statistical differences (P>0.05).

The fish corporal composition as the table 3.4 demonstrate had significant statistical differences between PAP3 and PAP4 for ash and fat parameters. The protein and energy percentage does not show any difference for all the groups.

**Table 3.5 –** Retention and gain during the 112 days feed trial.

| | GAIN (g) | | | RETENTION (%) | | |
|---|---|---|---|---|---|---|
| | PRO | FAT | Energy | PRO | FAT | Energy |
| **CTRL** | 1123.33±19.63 | 557.80±9.75[ab] | 429.00±7.5[a] | 41.84±0.68 | 49.36±0.79[a] | 26.94±0.33[b] |
| **PAP2** | 1051.16±8.92 | 567.74±4.59[ab] | 418.11±3.46[a] | 42.25±0.25 | 39.71±1.16[ab] | 29.35±0.12[a] |
| **PAP3** | 1056.46±16.79 | 429.16±7.26[b] | 368.69±6.02[b] | 38.52±0-87 | 34.28±0.77[b] | 25.82±0.49[b] |
| **PAP4** | 1068.45±37.56 | 657.45±20.55[a] | 454.26±15.04[a] | 41.33±1.97 | 43.76±1.76[ab] | 30.81±1.33[a] |

The table features each group average retention values (g) of the protein (PROT), fat (FAT) and Energy (kj/g DM). The table also has values representing the gain (%) in protein, fat and energy with standard deviation (n=2) and statistical differences (P>0.05).

The retention and gain values featured significant statistical differences (Table 3.5). In the fat gain, the CTRL and PAP2 groups showed no differences in relation to PAP3 and PAP4 groups and PAP3 showed differences in relation to the PAP4. In the energy retention PAP3 featured differences in relation to all other groups. In the fat retention values we got significant statistical differences between CTRL and PAP3 and in the energy retention values, CTRL and PAP3 showed differences in relation to PAP2 and PAP4

# 4. Discussion

There was seen in the table (3.1 and 3.2) that the feeds formulations showed some differences in their compositions and were not isoproteic, isolipidic and isoenergetic. For this reason there was expected some differences in the end of the trials for each group. It was satisfactory to see that the fish growth did not show any differences in relation to the CRTL diet (Table 3.3) and it was similar during all 112 days checkpoints (graphic 3.1). That indicate the components replaced in feed formulation did not affect the normal growth on the rainbow trout. We can relate the uniform growth in this trial to the level of replacement made. In this trial we did not aim to test the fish limitations in relation to high replacements of terrestrial animal rendered products, for that reason the feeds were formulated with low changes in the relation to the commercial feed (CTRL). The objective was to make a more economic and sustainable feed with actual use to the aquaculture industry.

When we try to go a little deeper in the replacement of fish meals and oils and all the common components used in the feed formulation, we need to surpass the digestibility barrier and nutritional equilibrium (EL-Haroun et al. 2009). The table 3.1 showed that we needed to supplement some amino acids to assure the nutritional wellbeing of this species. This amino acid supplementation result of the known issues related to the PAP's used in this feeds. For example, the protein quality loss in the blood meal processing and the feather meal low quality protein profile (Nengas et al. 1999; El-Haroun & Bureau 2007). Sometimes even with this supplementation it is impossible to maintain a similar performance to the commercial feeds, for example the trial made by EL-Haroun et al. 2009 where even with amino acid supplementation in some high level replacement diet, he had statistical differences in the rainbow trout growth. Although this fact, he could replace successfully and without any growth differences some feeds with meat bone meal, feather meal, blood meal and poultry by-products meal. The results obtained in our trials are in accordance with EL-Haroun et al. 2009 work. The amino acid supplementation failure in his work could be related to amino acid digestible composition and the limitation in other amino acids that were not predicted. The results obtained in our trial indicate that our amino acid supplementation appear to be the indicated.

Only recently the PAP’s and other terrestrial animal rendered product ban was lifted by the EU, this fact left us only with a handful of people working at this moment in this feed replacement projects, especially with rainbow trout. On the other hand there were a lot of studies made in this area on seventies and eighties. But the improvement made in animal products processing make those works outdated. Nowadays we have better quality and better digestibility on these products and subsequently better results. For this reason is more accurate to compare our results in relation to the work made by recent authors such Bureau et al. 1999 and EL-Haroun et al. 2009, Baboli et al. 2013 specially because they work with the same species. The results obtained in our work are mostly in accordance with their work to the same types of rendered proteins and incorporations in the feeds. Other authors working with other species such Chinook salmon, silver sea bream, Australian snapper, gilthead sea bream, red drum, Nile tilapia, obtain similar result using poultry products such as feather meal and poultry fat (Fowler 1991; El-Sayed 1998; Quartararo et al. 1998; Nengas et al. 1999; Kureshy et al. 2000).

On the other hand in other studies and even in the same studies mentioned above the results were not so satisfactory (Yanik et al. 2003; EL-Haroun et al. 2009; Bahrevar & Faghani-langroudi 2015). These results were affected by the high level replacement of fish meal by rendered animal proteins or even by the experience design and methodology that led to a decrease in the growth.

In these replacement trials there is a concern with feed palatability and the feed rejection that could lead to a decrease in voluntary feed intake. Normally, blood meals and poultry products have a good palatability, especially when used in carnivorous fishes such as rainbow trout, the major concern come from the feather meal usage (D. P. Bureau et al. 1999; Baboli et al. 2013). In this work we did not saw differences in this parameter and we can assume that the feather meal used did not affect our feeds palatability.

We did not see differences in feed conversion rate even during the 112 days checkpoints (graphic 3.2). Similar results can be found in Bureau et al. 2000; Yanik et al. 2003 and Baboli et al.2013, that use poultry by products and blood meal in their grow trials with rainbow trout. Once again we need to pay attention to the level of substitution made. The same authors verify that for high replacement rates the values differ from the control and some such as Bahrevar & Faghani-langroudi 2015 did not obtained satisfactory feed conversion rates results even in low levels of replacement with blood meal and poultry by products.

The hepatosomatic index showed no differences. This parameter was measured to assess the fish health conditions. Most related studies do not give importance to this parameter, but is important to verify if the rendered animal products have some influence in this species liver and consequently in their wellbeing. We can assume that the liver from the tested fishes did not suffer any major differences with the rendered animal products usage in their feeds and therefore the fishes should be healthy.

In protein efficiency ratio parameter (table 3.3), we start to see some statistical differences between groups. The first obvious consideration is the substitution made in the feeds. Although this fact appear to be the obvious one, the bibliography did not confirm that the rendered proteins used in our trial have influence in the PER, at least at the level of substitution made (Baboli et al. 2013). Other consideration to take is the quality of the protein used, that is questionable due to the feather meal usage (Bureau 2006; Hu et al. 2013). But this fact should in theory, affect the growth parameter as well and that was not verified in our trials. Taking in consideration that the feed were different in protein percentage we can assume that these differences should be related to the amount of protein available. The differences seen were between PAP2 and PAP3 diets. The protein percentage of these 2 diets are 37.8% and 41.2% (table 3.2) respectively and corresponds to the lower and higher protein content diets. For this reason and because neither of the diets showed differences in relation to the CTRL we can assume that this result were not related to the rendered terrestrial animal protein usage. In a more deeper analyses we can assume that PAP4 could show differences because it have 38.6% of protein, but the range of values used in statistical analyses could not differentiate this diet from the other higher protein percentage diets, like CTRL and PAP3 (table 3.2).

More important than growth efficiency is the fish quality in the end of the production. There is a constant concert related to the replacement of feed components because it could change the corporal constitution. Is important to maintain the fish protein and lipid profile that give fishery and aquaculture industries products, the healthy tag. For this reason we should evaluate the fish condition.

In fish condition parameters (table 3.4) we can find some differences between groups corporal composition for ash and fat percentage. In the ash content the differences were found between PAP3 and PAP4 diets. Studies demonstrate that some rendered terrestrial animal products have lower content in ash in comparison to the normal components used in commercial feeds (Bureau et al. 2002; Bureau 2006). This value depends on the mineral content and all the components that did not burn in analyses process. The use of bone meal feather meal and other high mineral content components can increase this value. In this case we see differences between two feeds that were formulated using terrestrial animal meals and fat. Although the feather meal was used in all the test feeds, we see that the initial content in ash on these feeds is lower than in CTRL diet as expected. It was expected see differences in final carcass on ash content between the test diets and CTRL only. The reason to explain the difference between PAP3 and PAP4 can be related to the fat content, which actually showed statistical differences to the same diets. We can see that PAP4 have a higher fat content in the diet and in final fish body composition, on the contrary PAP3 have lower fat content in the diet and in final fish body composition. For this reason fish fed on PAP4 diet assimilate more fat content than PAP3 fishes (Bureau et al. 2002; Webster & Lim 2002). Fat or lipid content is known to be highly volatile (Bureau 2006). For this reason when the fish body composition analyze was made we obtain lower ash content to PAP4 and higher to PAP3. This simple conclusion seems to be the better explanation to this difference. We need to be aware that the ash content is not an important factor to the aquaculture industry, because the mineral content in feed, as exception of phosphorous and other secondary mineral, is not relevant to the fish growth and fish have the capacity to extract some minerals from the water (Webster & Lim 2002). Although this explanation seems more likely, we can question about this conclusion cause in table 3.2 we can see that PAP2 diet have higher content in fat and therefore we can assume that it should have low content in ash on fish body composition. It is plausible that this result is related, to the slightly higher ash content in the feed formulation in comparison to PAP4 feed. Although the replacement made in test diets show few differences between themselves, the components replaced to introduce our animal rendered products were not the same in all diets. This could affect the ash content in the feed and lately on fish body composition.

The fat statistical differences found between PAP3 and PAP4 fish body composition should not be related with rendered terrestrial animal products usage. The differences seen could be related with fat content in the both diets. As said above PAP4 diet had more fat percentage than PAP3 diet, 22.4% and 18.8% respectively. The fact that the same type of terrestrial processed fat was used in the feed formulation and the absence of differences in relation to the CTRL fishes only lead to this conclusion. We need to be aware that the CTRL diet has low fat content and following the same theory we should have seen differences in relation to this diet. The explanation to the inexistence of this difference seems to be related to the CTRL diet high content in protein and energy (40.6 % and 24.1% respectively) (table 3). The metabolism of carnivorous fish like rainbow trout use protein primarily and fat secondarily as source of energy, however there is important to pay attention to the energy available in the feed. For this reason we see that the commercial feeds have an adequate quantity of energy in their formulation, preferably one ration of 8/10 kcal of energy for each gram of protein (Gatlin 2010). This energy value ensures a higher protein and fat usage efficiency for the fish growth. Supported by this explanation we can assume that although the CTRL diet showed to have low fat percentage in relation to PAP4 diet we did not obtain differences, because the energy and protein contained in the diet assure that the fat was not used in the normal fish metabolism but in the growth and deposition on fish body.

We did not see significant statistical differences on the energy parameter for the body composition. We can assume that the rendered animal product used and the differences in the feeds energy composition did not influence the final body energy ratio. This result is in the accordance with Bureau et al. 2000 to similar rendered animal product. The energy values can be influenced by the protein and fat profile presented in the body composition (Gatlin 2010). But despite the differences seen in the fat parameter for the body composition we can assume that those values did not influenced the energy parameter. We also need to be aware that the energy represented in the table 3.2 is the gross energy and not all the energy is digestible (Gatlin 2010).

In the gain values (table 3.5) we got differences between PAP3 and PAP4 diets on the FAT parameter, the same differences found on the body composition. The gain is obtained by the difference of group initial body composition and group final body composition in the relation to the group weight. The result reflects in a way the fish final body composition percentages. For this reason the explanation for these differences on fat gain seems to be the same as the body composition. Energy gain showed differences in PAP3 in relation to the other groups (Table 3.5). This value is related to the energy in the fish body composition, which was the lowest in all the groups. The energy gain reflects the energy digestible in the feeds and therefore the protein and lipids digestibility contained in the feeds (D. P. Bureau et al. 1999; Gatlin 2010). For this reason we can assume that the PAP3 have less digestible energy in its components used. On the other hand we cannot relate this to the rendered animal products usage, because the substitution on the feeds had little differences between them and for this reason we should see differences in the other test groups as well.

On the retention results we verify once again differences for FAT percentage (Table 3.5), but this time, there are differences in CTRL diet in relation to PAP3 diet. CTRL have a higher retention value due to the fact that it had low fat content in diet but kept a satisfactory value in body composition. CTRL diet had a higher energy composition in relation to the test feeds for this reason we see, as mentioned above, that the fish feed on this diet have a better efficiency on retain protein and fat (Sanz et al. 2000; Bureau et al. 2002; Boujard et al. 2002).

We can assume that once again the PAP3 group low fat retention is related to the low content on fat in diet that led to this low retention result. But as we see above, we suspect that PAP3 have some digestible problems in its components due to the low energy gain. We can also see that PAP3 had the lowest fat gain and fat in body composition. The fat is the major energy giver component to overall energy in the carnivorous fish feed (Gatlin 2010). For this reason we can assume that the lack of digestibility on PAP3 could be related to the fat source. The protein digestibility can also change the energy parameter, but in this case seems not be the explanation, since there is no differences on proteins parameters in gain and retention calculation. However CTRL and PAP3 did not shown differences on energy retention and PAP2 and PAP4 differ from them (table 3.5). These differences suggest that after all, PAP3 components seem to be more digestible than we thought with the previous values of fat parameters due it similarity to CTRL. Moreover the rendered animal products digestibility appears to have no effect in these energy retention results, because PAP2 and PAP4 obtain higher values than the CTRL. Independently of the fat percentages or if it shows statistical differences or not, we can see in table 3.5 that all test diets have lower fat retention in relation to CTRL diet. This lack of retention capacity could be related to the rendered animal products used in the feeds formulation and is digestibility

The digestibility trials of these diets were not done so we cannot accurately make a conclusion. It's known that exists some factors that can affect fat components such as the nutritive value of the fats with different fatty acid profiles at different water temperatures (Bureau et al. 2002; Tocher 2003). It was documented that lard and tallow (lipid source with high saturated fatty acids) were affected by the water temperature, especially at lower temperatures because lard and tallow have a high melting point (Cho & Kaushik 1990). However the literature says that the lard and tallow fat profile differ greatly from poultry fat and oils that we use in our trials (Bureau et al. 2002; Emery et al. 2014).

In the work made with Atlantic salmon by Emery et al. 2014, where he replace the poultry oil contained in a commercial diet by tallow, he got a decrease in the fat digestibility, directly related to the amount of replacement. He link this decrease to the tallow melting point and the relation of saturated fatty acids (less digestible) with monounsaturated and poliunsaturated fatty acids amount. He also found no differences in the growth, similar to our results. He assumed by these results that poultry fats had less problems related to the melting point and fatty acid profile. Other authors say that lard and poultry fats have rich content in saturated fatty acids, however the poultry fats have a better profile and amount of monousaturated fatty acids (Bayraktar & Bayır 2012).

The fact that poultry fats have a good amount of monousaturated fatty acids may suggest that this component is more digestible. Bureau et al. 2002 in is review verify that some authors indicate that exist some synergies between poliunsaturated fatty acids and highly saturated lipids. This synergy suggests that the saturated fatty acids have a better digestibility when there is a good amount of mono and poli unsaturated fatty acids. When highly saturated lipids are used almost exclusively in the feed formulation we see a great decrease in digestibility. For this reason when aquafeeds containing rendered animals fats are formulated we need to be aware of this synergy and when this equilibrium is not possible we should supplement monounsaturated and poliunsaturated fatty acids in this formulation to acquire a good digestibility. This information may not explain directly what really happened in our retention results but may give hint to the factors behind this question.

We do not have the information about the fatty acids profile contained in the feeds and in the body composition. Those values would be of most importance to explain the low fat retention for the tested feeds and to assure if the feeds and fish have the fat profile necessary to the aquaculture industry and consumer, with all fatty acids required for fish quality and healthy factors. Although in all tested diets we do replace fish meal and oil, we also replace some vegetable products (table 2.1). These substitutions were not made in the same components and for this reason is hard to attribute the differences found to the vegetable components equilibrium or to the rendered animal protein usage. To complement this research it would be important to do an organoleptic test with objective to assure that the fish have the pigmentation and flavor required to be commercialized.

# 5. Conclusion

The results of this work confirm that the rendered terrestrial animal products used in this work can be used in aquafeed for rainbow trout without any negative effect in the fish growth for the amount of replacement made. Some kind of amino acid supplementation however is needed to assure these results. Moreover the few problems related to these products can be highly profitable by their low price and constitute an economic viable solution to replace fish meal and oil resulting in very significant savings. These results also bring some new formulations that can be adapted in aquaculture industry giving new options for this production sector. We need to be aware of the fat digestibility that showed to be the more problematic aspect in this work. More studies need to be done to acquire information about the effects of this problem on further feed formulations and on fish body fat profile.

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